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Research Article

Occurrence of gregarine parasites of *Tenebrio molitor* (Coleoptera: Tenebrionidae) in Georgia and Türkiye

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Abstract

Gregarines, which belong to the parasitic protozoa, have a variety of negative effects on their hosts. Therefore, it's important to report this pathogen for the first time from a new insect population in Tbilisi, Georgia. Several life stages of the gregarine were found in the midgut lumen of the yellow mealworm, *Tenebrio molitor*, a pest species of grain and stored food products, which is also gaining attention as a potential sustainable food source. Forty samples from both larvae and adults were dissected, and their intestines were examined under a microscope, demonstrating that both were infected by the parasite. Several life stages of the gregarine parasite were found in the midgut lumens of these mealworms, with the largest trophozoite observed being 67.9 µm in length. Gregarine infection rates in *T. molitor* larvae were found to be higher than in the adults. Twenty-nine of the 40 examined larvae were infected (72.5%) compared to seven of the 40 examined adults (17.5%). These results suggest that *T. molitor* larvae are more susceptible to gregarine parasite infection than adults. Our study contrasts with a similar study in Antalya, Turkey, where no gregarine infection was observed in either larval or adult *T. molitor*.

Key words: apicomplexan protists, Caucasian coleoptera, distribution, infection rate

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Introduction

The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), is a critically important insect species with both beneficial and detrimental role for humans; it can therefore be described as a "beneficial pest" (Vigneron et al. 2019). As a pest species, it infests stored cereal products (Bjørge et al. 2018), but it is also one of the most valuable and high-nutritional food insects; it's used in the animal and human food industries and consequently is currently also being promoted as a beneficial insect. It's capable of degrading polystyrene and plastic waste (Bjørge et al. 2018; Vigneron et al. 2019), and it's also used as a model organism in scientific research (Martynov and Brygadyrenko 2018; Brai et al. 2023). The high-nutritional aspect of *T. molitor* makes it an attractive option for the mass production of food. However, the high-density rearing con-

ditions associated with mass production may increase the risk of disease and infections by parasitic symbionts and promote their spread within these insect colonies. These, in turn, may be passed to humans and animals consuming the mealworms as food (Siemianowska et al. 2013; Vigneron et al. 2019; Slowik et al. 2023).

Gregarines are protozoans belonging to the phylum Apicomplexa and are parasites of many different groups of invertebrates. The effects of gregarines on their hosts have been the subject of numerous experimental studies and have been shown to be both detrimental and beneficial. Their negative effects include arresting development, causing mortality, and inhibiting reproduction in their hosts (Lazurska and Brygadyrenko 2024). Gregarines can also be beneficial, increasing host growth, fitness, and longevity (Slowik et al. 2023). Multiple gregarine species have been recorded in the digestive tract of *T. molitor*.

As *T. molitor* is both harmful to products in storage and beneficial in the mass production of a primary protein source for human and animal nutrition, it is regarded as a target organism for the study of gregarine pathogens in different geographic populations. In this study, we investigated the presence of gregarines in two populations of *T. molitor* obtained from Georgia and Türkiye.

Material and methods

Samples of adult and larval yellow mealworms, *T. molitor*, were provided from insect-rearing facilities in Tbilisi, Georgia, and Antalya, Türkiye. Forty samples of both larval and adult individuals were dissected in a physiological solution, and their intestines were examined under a microscope for protozoan parasites at a magnification of 10x to 1000x (Yaman, 2020). When a parasitic infection was observed, part of the material was used to prepare Giemsa-stained smears. For this, the slides were air-dried and fixed with methanol, then stained with a freshly prepared 5% solution of Giemsa stain and re-examined under the microscope. The parasite's life stages detected by light microscopy were measured and photographed using a microscope with a digital camera and a Soft Imaging System. The following structures were measured: length of epimerite (LE), length of deutomerite (LD), length of protomerite (LP), total length (TL), width of deutomerite (WD), width of protomerite (WP), the ratio of the length of the protomerite to the total length (LP:TL), and the ratio of the width of the protomerite to the width of the deutomerite (WP:WD).

Results and discussion

During the study, gregarine infection was only observed in the Tbilisi (Georgia) population of *T. molitor*. Several life stages of the gregarine parasite were found in the midgut lumens of both the larvae and adults of the yellow mealworm (Figs. 1, 2, 3). Each trophozoite was elongate with a bud-type epimerite (5.5 µm) (Fig. 1). The largest trophozoite that could be observed was 67.9 µm. The average measurements of the trophozoite are given in Table 1.

Sporadins found in the midgut lumen of the host were solitary (Fig. 2). The septum between the protomerite and the deutomerite was clearly distinct. The deutomerite was ovoid to ellipsoid, with a spherical nucleus, 10.3 µm in average diameter. The average measurements of the sporadins are given in Table 1.

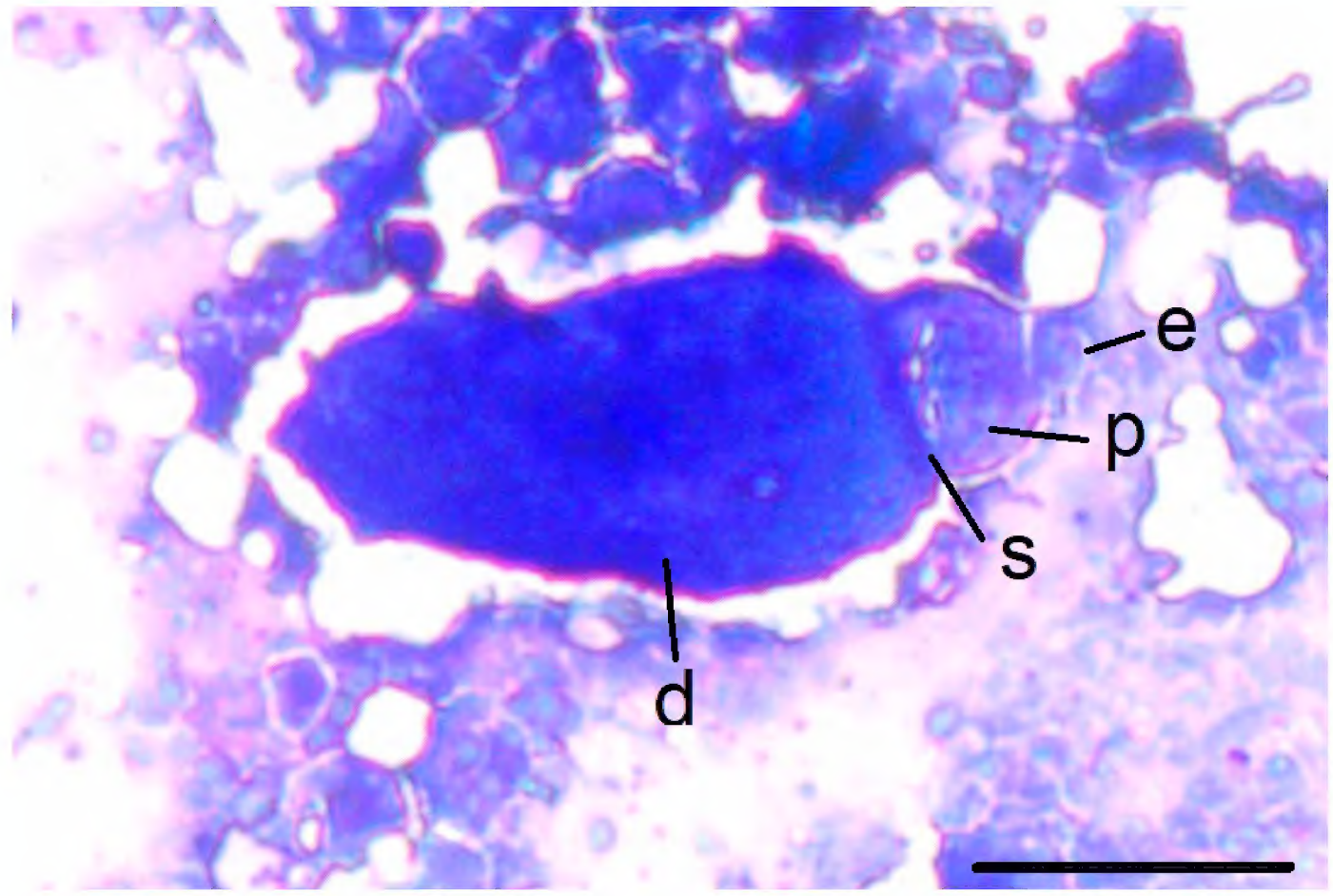

Figure 1. Trophozoite of gregarine parasite in *T. molitor*. **e**: epimerite; **p**: ccccprotomerite; **d**: deutomerite; **s**: septum (Bar: 25 µm).

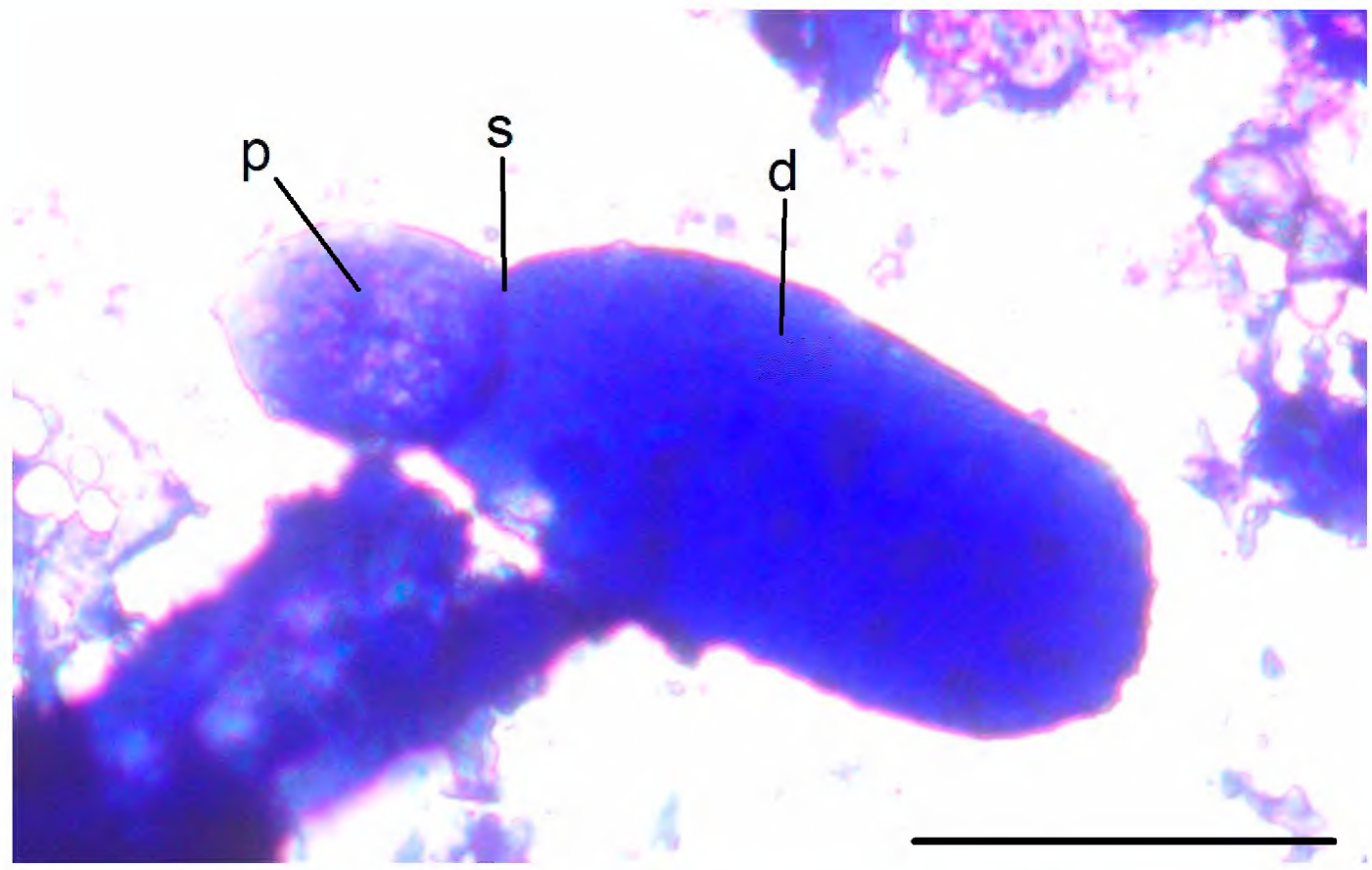

Figure 2. Solitary sporadin of gregarine parasite in *T. molitor*. **p**: protomerite; **d**: deutomerite; **s**: septum (Bar: 50 µm).

Sporonts were biassociative and ovoid to ellipsoid. The primite had a conical to hemispherical protomerite. The septum was clearly distinct. The deutomerite was ovoid (Fig. 3). The measurements of the associations are given in Table 1.

As seen in Table 2, gregarine infection rates in *T. molitor* larvae were higher than in the adults. Twenty-nine of the 40 examined larvae were found to be infected, an infection rate of 72.5%. However, only seven of the 40 examined adults were found to be infected, an infection rate of 17.5%. The results indicate that *T. molitor* larvae are more susceptible to the gregarine parasite than

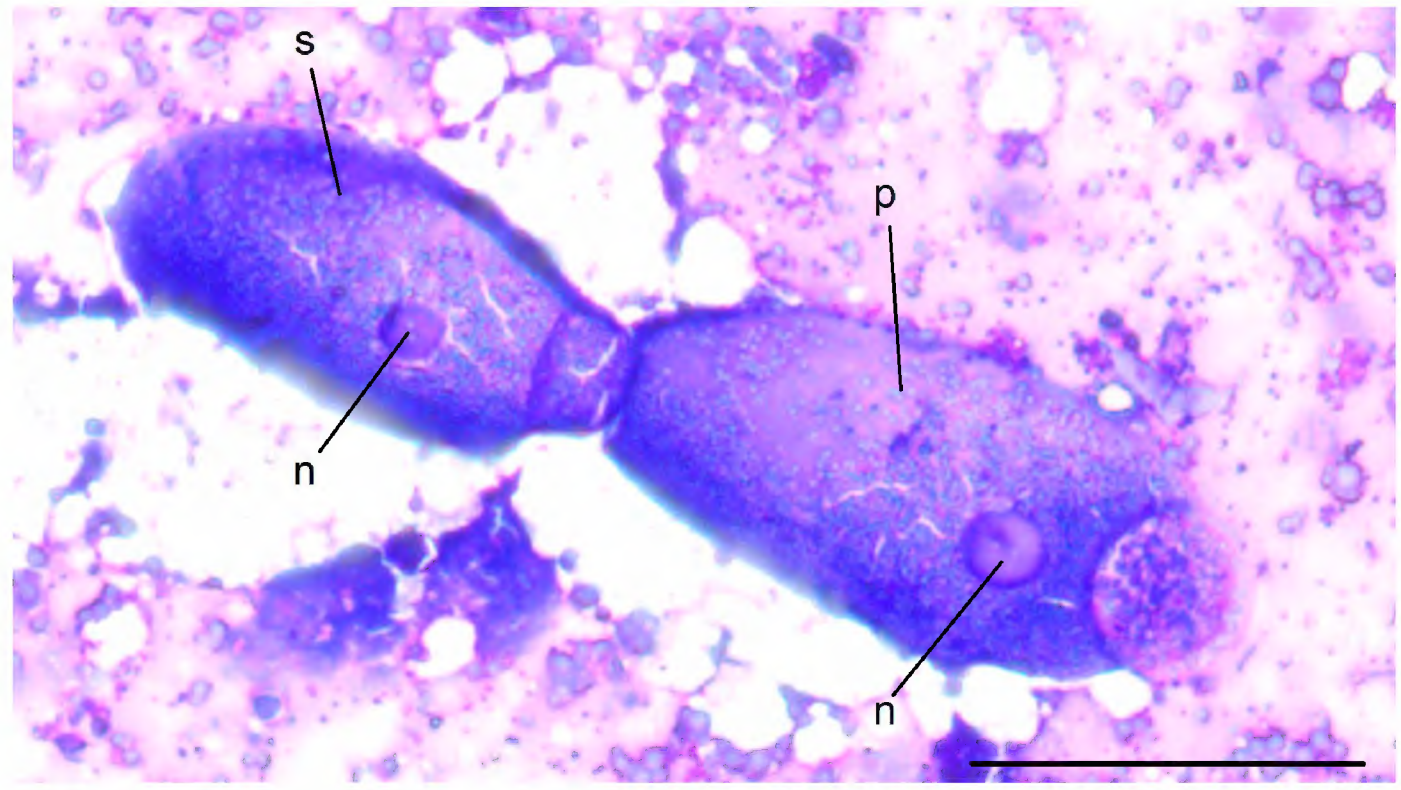

Figure 3. Association of gregarine parasite in *T. molitor*. **p**: primite; **s**: satellite; **n**: nucleus (scale bar: 50 µm).

the adults. In contrast, there was no gregarine infection in either the larvae or adults of *T. molitor* from the Antalya, Türkiye, population.

According to Clopton et al. (1991) and Slowik et al. (2023), members of the subclass Gregarinasina, commonly known as gregarines, including eugregarines and neogregarines, are a well-studied protist group infecting *T. molitor*. In a recent review on the harmful and beneficial symbionts of *T. molitor* by Slowik et al. (2023), amongst the eugregarines, four species are known to infect this

Table 1. The measurements of trophozoites, sporadins and associative forms of gregarine parasite in T. molitor in mm (**a**: mean and standard deviation; **b**: minimum and maximum). **TL**: total length; **LP**: length of protomerite; **LD**: length of deutomerite; **WP**: width of protomerite; **WD**: width of deutomerite; **LP:TL**: ratio of the length of protomerite to total length; **WP:WD**: ratio of the width of protomerite to the width of deutomerite.

	TL	LP	LD	WP	WD	LP:TL	WP:WD
Trophozites	62±8.4[a]	10.2±1.2	47.9±3.6	15.6±1.8	21.7±5.9	6.1±0.1	1.4±0.2
	(56–67.9)[b]	(9.3–11)	(45.3–50.4)	(14.3–16.8)	(17.5–25.9)	(6–6.2)	(1.2–1.5)
Sporadins	181.9±57.2	26.7±5.8	154±55.9	29.9±7.3	59.9±14.9	7±2.4	2±0.3
	(101–250)	(21–37.1)	(78–229)	(19.6–46)	(39.6–92)	(3.3–11.4)	(1.6–2.3)
Primite	120.2 ± 42.7	20.2 ± 5	99.9±39.9	24.5±4.1	44.6±8.3	6.±1.9	1.8±0.3
	(82.2–192.9)	(15.2–29.6)	(64.8–172)	(19.7–31.2)	(33.4–58.3)	(4.6–9.3)	(1.5–2.3)
Ilite	101.5±38.1	15.8±3.4	86.1±35.4	23±5.3	39±6.1	6.4±2	1.7±0.3
	(53.5–165)	(10.3–20.8)	45.4–147.5)	(14.5–29.7)	(28.5–44)	(5–9.8)	(1.4–2)

Table 2. Parasitism of the yellow mealworm *Tenebrio molitor* by gregarine parasite in Tbilisi (Georgia) and Antalya (Türkiye).

Locations	Larva			Adult			Total average (%)
	Examined samples	Infected samples	Infection rate (%)	Examined samples	Infected samples	Infection rate (%)	
Tbilisi (Gerogia)	40	29	72.5	40	7	17.5	45
Antalya (Türkiye)	40	0	0	40	0	0	0

species: *Gregarina cuneata*, *G. polymorpha*, *G. niphandrodes*, and *G. steini*, of which *G. cuneata* appears to be the most impressive and complex among them.

A well-documented comparative study by Clopton et al. (1991) of gregarine species infecting *T. molitor* has described their morphology, general appearance, morphological measurements, and ratios. Using these descriptions, the gregarine parasites from the *T. molitor* in our study are similar to the descriptions given of both *G. cuneata* and *G. niphandrodes* in terms of their body morphology and general appearance. However, they differ from the description in both morphological measurements and ratios.

Clopton et al. (1991) also report that *G. polymorpha* and *G. steini* are only found in the larvae of *T. molitor*, whereas *G. niphandrodes* was reported only from adults. *Gregarina cuneata*, in contrast, is reported to infect both larvae and adults of *T. molitor* (Stein, 1848; Schneider, 1875).

As the gregarine parasites found in this study were in both larvae and adults of *T. molitor*, our results suggest it is possible that more than one gregarine species might be infecting the Georgian population of this mealworm.

Conclusion

Gregarines parasitize many groups of insects (Yaman, 2002, 2004, 2007), and their effects on their hosts have been the subject of numerous experimental studies. At times, they exhibit effects that are both detrimental and beneficial to their hosts. *T. molitor* is both harmful to storage products and beneficial as a mass-produced primary protein source for human and animal nutrition, making it a target organism for studying its gregarine pathogens in different geographic populations. Our results show that more than one gregarine species may be infecting the Georgian population of *T. molitor* or can be found in the same *T. molitor* host. There remains the possibility that a new gregarine species might be identified from the *T. molitor* populations in Georgia. Therefore, further characterization of the gregarines in Georgian populations of *T. molitor*, including molecular phylogeny studies, should be carried out, and the presence and distribution of gregarines in other *T. molitor* populations in Georgia should be documented.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

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Author contributions

MY: conceptualization; validation; writing, reviewing, writing the original draft, supervision; MK: methodology, resources, and editing the original draft; TSG: methodology; editing the original draft.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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